RELATIONSHIP BETWEEN NUCLEAR VOLUMES, AMOUNT OF INTRANUCLEAR PROTEINS AND DESOXYRIBOSENUCLEIC ACID (DNA) IN VARIOUS RAT CELLS

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The concept that increase in size of nuclei and cells within one tissue is directly correlated with an increase in chromosome number or chromosomal mass has become more or less accepted in cytology (Hertwig, 1934, 1939).

But Montgomery (1910), Bowen (1922) and Schrader (1947) have shown that in different sizes of spermatocytes in pentatomid testes, the chromosome number and chromosome size are identical in the different sized cells. Recently, Schrader and Leuchtenberger (1950), using quantitative cytochemical methods, presented evidence that in the pentatomid Arvelius there exists a direct correlation between three sizes of nuclear volumes of spermatocytes (200, 400, 1600 cubic microns), and the amount of contained proteins, while the amount of DNA was the same in these nuclei. On the basis of these results, the assumption was made that changes in nuclear sizes in a tissue are due mainly to alterations in protein and water content, and not due to different amounts of DNA.

The present communication presents further evidence on a parallelism between size of nuclei and amount of intranuclear protein within one tissue, but points out that such a correlation does not apply to a tissue containing cells with different synthetic activities, such as the parietal and chief cells of the gastric mucosa.

Material and Methods

Rat tissue was fixed immediately after death in 50 per cent formalin and Carnoy and cut at different thicknesses, according to the requirements of the investigation. The estimation of DNA and protein was done by the cytochemical and photometric microscopic methods previously described (Schrader and Leuchtenberger, 1950; Pollister, 1950).

Results

As can be seen from the data in Table I, there occur three distinct classes of DNA, namely 3.3, 6.6 and 12.8, in liver nuclei of the rat, an observation which is in agreement with the findings of Ris and Mirsky (1949) and Leuchtenberger, Vendrely and Vendrely (1951). But it is also evident that in contrast to the nearly exact multiples of DNA in the nuclei (1, 2, 4), the nuclear volumes do not show such a multiple relationship, but vary over a large range with intermediate values. It can be furthermore seen that there is no correlation between nuclear size and amount of DNA per nucleus; within each DNA class the size of nuclei varies considerably. For instance, among 36 nuclei with a DNA content of 6.6 per nucleus,

TABLE I

Relationship between nuclear volumes, amounts of intranuclear proteins and desoxyribosenucleic acid of rat liver cells*

	Mean Amount of DNA per Nucleus = 3.3 ± 0.08			Mean Amount of DNA per Nucleus = 6.6 ± 0.24			Mean Amount of DNA per Nucleus = 12.8 ± 0.50		
Mean nuclear volumes in cubic microns	62.0	153±17	289±27	285±20	455±30	732±78	376	637	1138
Number of nuclei measured	3	20	6	18	13	5	4	4	6
Mean amount of protein per nucleus	1.9	4.6±0.23	8.7±0.3	8.6±0.1	13.7±0.5	22.0±1.2	11.3	19.0	34±1.4

* Amounts expressed in arbitrary units.

18 showed a nuclear volume half or less than that of the remaining nuclei. The independence of nuclear size and of DNA content is also obvious if one compares the nuclear volumes of one DNA class with those of another DNA class; *i.e.*, 6.6 DNA per nucleus occurs in cells with nuclear volumes which are as small as those of the 3.3 and as large as those of the 12.8 DNA class. The occurrence of nuclear volumes in nonmultiples is in agreement with the data of Biesele (1944) on volumes of rat liver nuclei.

The picture is considerably different if one considers the amount of protein per nucleus in relation to nuclear size. It is evident that there exists a direct correlation between both; any increase in nuclear volume is accompanied by a corresponding increase in amount of intranuclear protein. In order to see whether such a close relationship between size of nuclei and amounts of contained proteins would hold in tissues in which cells with different functions occur, we examined the gastric mucosa of the rat. It is known that there the chief cells produce a protein, namely a precursor of pepsin, while the parietal cells produce HCl. In the following Table II, the results are presented.

On the basis of the data in Table II it is evident that the amount of DNA per nucleus is the same in chief cells and parietal cells. It can be further seen that

TABLE II

Relationship between nuclear volumes, amounts of intranuclear proteins and desoxyribosenucleic acid of rat gastric mucosa cells*

Type of Cell	No. of Nuclei Measured	Nuclear Vol. in Cubic Microns	DNA per Nucleus	Mean amount of Protein per Nucleus
Parietal Cell	19	57±4	2.9±0.1	1.0±0.1
Chief Cell	20	44±2	3.1±0.1	1.9±0.09

* Amounts expressed in arbitrary units.

though the nuclear volumes of the parietal cells are larger than those of the chief cells, the latter contain a larger amount of protein per nucleus than the parietal cells. Therefore, the strict parallelism between amounts of proteins and nuclear size, as found in liver cells or spermatocytes, does not necessarily hold for cells which differ in the nature of their synthesizing activities, such as the chief and parietal cells of the gastric mucosa.

Discussion

The close correlation between amount of intranuclear proteins and nuclear volumes, and the independence of the amount of DNA from the latter in rat liver nuclei is in good agreement with the previous work of Schrader and Leuchtenberger (1950) concerned with proteins and DNA in spermatocytes.

A similar correlation between amount of proteins and nuclear sizes in oocytes of mice was recently reported by Alfert (1950). The striking parallelism between amounts of proteins and size of nuclei in all these tissues is based on the fact that the concentration of proteins stays the same in all nuclei, whether the cell decreases or increases in size.

But these observations do not apply to all the tissues of the same animal, as can readily be seen from the results in cells of the gastric mucosa. It seems that the correlation exists only if cells actually have the same metabolic function as, for instance, the cells of the parenchyma of liver or the spermatocytes in the testes. However in a tissue in which cells occur having different synthetic activities, the concentration of the proteins may vary. In the gastric mucosa, the protein-producing chief cells show nearly twice the amount of protein per nucleus, though their nuclear volume is smaller than that of the HCl-producing parietal cells. These results are in good agreement with Caspersson's work (1950), who, on the basis of ultraviolet studies, reported considerably larger amounts of proteins and ribosenucleic acid in chief cells than in the parietal cells.

Whether it can be assumed generally that variable concentrations of proteins in different cells of the same tissue indicate differences in protein synthesis cannot be decided at this time. But the possibility is now opened that the cytochemical photometric determination of proteins in individual cells may allow such a differentiation.

A comparative study of the protein content of nuclei of normal and abnormal cells is now in progress.

Summary

Cytochemical photometric microscopic determinations of proteins and desoxyribosenucleic acid in cells of rat tissues gave the following results:

1. A direct correlation exists between different sizes of nuclei and amounts of intranuclear proteins in rat liver cells, while the amount of DNA was found to be independent of nuclear size.

2. In the gastric mucosa, where cells with different synthetic activities (parietal and chief cells) occur, there is no parallelism between nuclear size and proteins. The chief cells have a larger amount of protein than parietal cells, while the amount of DNA is the same in both.

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